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Cellular Differentiation: The Violin Strikes up Another Tune

Dispatch

Michael A. Cook[1,2] and Mike Tyers[1,2]

A switch in cellular identity in budding yeast requires the ubiquitin-dependent elimination of pre-existing master regulators encoded by the *MAT* locus. Failure to disassemble the prior state not only impairs the cell type transition but imparts a hybrid cellular fate. This theme will undoubtedly arise in many developmental and disease contexts.

The agile mind of the late Ira Herskowitz inspired a generation of biologists, in no small measure through his metaphor of 'violins and orchestras' [1], meaning that the lessons learned from single-celled yeast would provide general principles applicable to multicellular organisms. The basis for cell type identity in yeast, solved in a thrilling molecular detective chase more than twenty years ago by Herskowitz and colleagues [2], revealed the first examples of eukaryotic 'master regulators', a term coined by Herskowitz. During vegetative growth, homothallic strains of the budding yeast *Saccharomyces cerevisiae* exist as one of three distinct cell types: two haploid mating types, **a** and α, and a non-mating **a**/α diploid formed by conjugation of the two haploid cell types. Haploid **a** and α cells each produce a specific pheromone, **a**-factor and α-factor, respectively, which causes cells of the opposite mating type to arrest in G1 phase, induce mating effector genes, and initiate polarized growth towards the mating partner. This response is achieved through activation of a MAP kinase cascade and a dedicated transcriptional program upon pheromone receptor engagement [3]. Diploid cells lack receptors and other components of the signalling machinery and so are unresponsive to pheromones.

These differences between cell types are controlled by the *MAT* locus, which encodes a small number of master regulatory genes. In **a** cells, **a**-specific genes are expressed constitutively (Figure 1A). In α cells, the transcription factor α1 activates α-specific genes, while the repressor α2 inhibits **a**-specific genes. In diploid cells, which express both *MAT***a** and *MAT*α information, the α2 repressor prevents **a**-specific gene expression, while the heterodimeric repressor complex **a**1–α2 inhibits α1 transcription, and thus α-specific gene expression, as well as expression of other haploid-specific genes.

Homothallic haploid yeast switch their mating type by gene conversion between the *MAT* locus and adjacent transcriptionally silent regions that bear **a** (*HMR***a**) and α (*HML*α) genes (Figure 1B), a process that depends on formation of a specific double strand break by the endonuclease encoded by the *HO* gene [4]. Mating-type switching occurs only in mother cells, and results in an immediate change in the *MAT*-directed transcriptional program, which is phenotypically manifest within a single cell cycle. This ingenious regulatory construction ensures that haploid yeast of opposite mating type are physically juxtaposed, such that a wild-type colony is composed predominantly of stress-resistant diploid cells (Figure 1C). The necessary rapid change of state presents an obvious problem, however — in order for a new transcriptional program to be established, the regulators of the previous program must be eliminated. A recent study by Laney and Hochstrasser [5] addresses the consequences of failure to disassemble the α-specific state after the mating-type switch.

The *MAT*-encoded transcription factors are rendered highly unstable *in vivo* through ubiquitin-dependent proteolysis [6,7]. In the ubiquitin system, the small protein modifier ubiquitin is activated and serially transferred along an enzymatic cascade, E1→E2→E3, then conjugated to the target protein, which is specifically recognized by its cognate E3 ubiquitin ligase [8]. Reiteration of the cycle builds a polyubiquitin chain on the substrate, targeting it to the 26S proteasome for rapid degradation. For the α2 protein, at least two distinct pathways for destruction exist: one depends on the E2 enzyme Ubc4 and an unknown E3, the other on the E2 enzymes Ubc6 and Ubc7, in conjunction with the E3 enzyme Doa10 (Figure 2) [9]. Whereas *ubc4Δ* and *doa10Δ* (or *ubc6Δ*) single mutant strains show only modest stabilization of α2, the protein is dramatically stabilized in *ubc4Δ doa10Δ* or *ubc4Δ ubc6Δ* double mutant strains.

To assess the consequences of α2 stabilization on mating-type switching, Laney and Hochstrasser [5] performed a pheromone confrontation assay, which measures the proportion of *MAT*α cells capable of responding to α-factor, signifying a switch to the *MAT***a** mating type. This switch is easily observable through the formation of a characteristic mating projection, called a shmoo. Consistent with the switching schema, the *ubc*/*doa* double mutants were defective for α-to-**a** mating type switching, whereas the single mutants were not.

As with any result of substance, the heart of the matter lies in the controls. Laney and Hochstrasser [5] first demonstrated that the kinetics of mating-type switching were indistinguishable in wild-type *versus ubc*/*doa* double mutants, by monitoring the rate of gene conversion at the *MAT* locus upon *HO* induction. They then constructed a strain in which the silent *HMR***a** locus was modified to include a constitutive GFP reporter instead of the *MAT***a***1* gene, such that upon induction of *HO*, only cells that undergo mating-type switching express the GFP reporter. Again, the frequency of mating-type switching in *ubc*/*doa* double mutants was indistinguishable from wild type. The best evidence against a general breakdown in the switching

[1]Department of Medical Genetics and Microbiology, University of Toronto, Toronto, Canada M5S 1A8. [2]Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada M5G 1X5. E-mail: tyers@mshri.on.ca

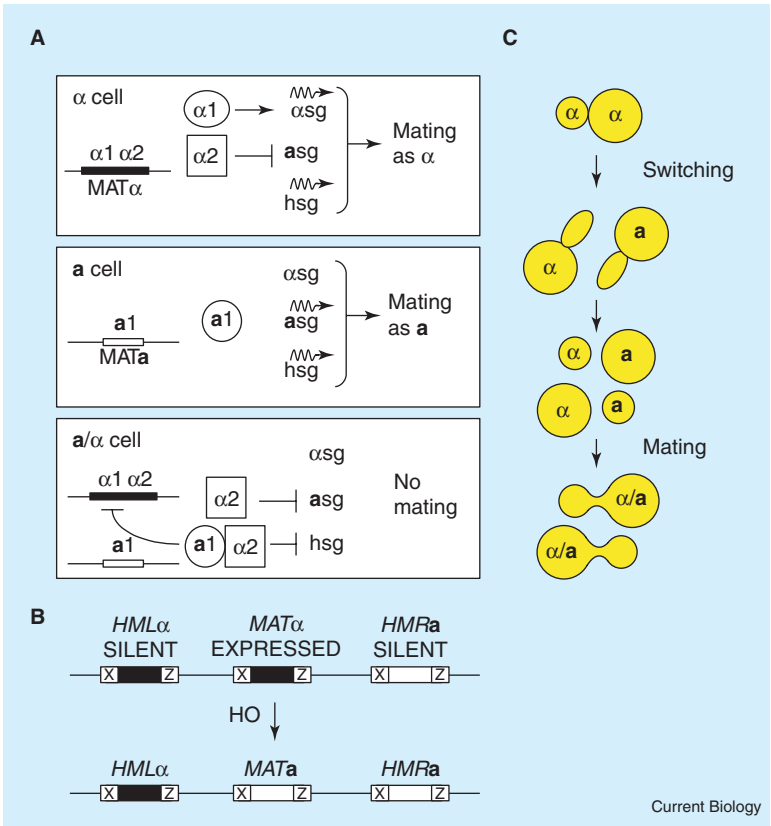

Figure 1. The specification of cell identity in *Saccharomyces cerevisiae.*
(A) Cell type is directed by three master regulatory proteins encoded by the *MAT* locus: **a**1, α1 and α2. These factors exert their effects through controlling expression of three gene sets: αsg, α-specific genes; **a**sg, **a**-specific genes; hsg, haploid specific genes. α1 activates transcription of α-specific genes; α2 represses **a**-specific gene expression, which is constitutive in **a** cells. In diploids, the heterodimeric **a**1–α2 repressor prevents both α1 and haploid-specific gene expression. (B) The *MAT* locus is flanked by transcriptionally silent copies of the α (*HML*α) and **a** (*HMR***a**) genes. Production of the HO endonuclease in mother cells causes a double-strand DNA break at the *MAT* locus, which is repaired by gene conversion using either *HML*α or *HMR***a** as a template, resulting in a switch in mating type. (C) Mating-type switching in a homothallic yeast colony. Mating-type switching in the mother cell at each division results in juxtaposition of cells of opposite mating type, which then conjugate to form the preferred diploid cell state. (Adapted from a review by Ira Herskowitz [1].)

machinery, however, was the asymmetry of the defect. That is, in an **a**-factor confrontation assay, *MAT***a** *ubc/doa* double mutants switched just as well as wild-type *MAT***a** cells. Taken together, these observations strongly suggest that the cell-type identity defect is caused by a failure to degrade a pre-existing regulatory component of the α cell. After ruling out other possible culprits, including the **a**-factor receptor, α2 stabilization remained the only possible cause of the inability to acquire the *MAT***a** identity.

Laney and Hochstrasser [5] logically proposed that upon α-to-**a** mating type switching in *ubc/doa* double mutants, the stabilized α2 protein not only inhibits **a**-specific gene expression but also combines with **a**1 to form inappropriate heterodimeric repressors normally found only in diploid cells [5]. The cumulative effect of these two mechanisms would be a failure to express the components necessary for **a**-factor pheromone response. In support of this idea, the authors were able to show that over-production of α2 partially inhibits the α-to-**a** switch in identity.

The argument was then sealed by the demonstration that deletion of **a**1 from the *HMR***a** locus, coupled with over-production of the α-factor receptor Ste2, which is normally repressed by α2, is sufficient to restore appropriate mating type switching to a *ubc4Δ doa10Δ* double mutant. These findings raise follow-on questions, including the means by which **a** cells eliminate **a**1 upon switching, and the means by which diploid cells eliminate the normally rock stable **a**1–α2 complex during sporulation [10]. An unresolved mechanistic issue is how α2 is ubiquitinated and eliminated at its cognate promoters, especially as Ubc6, Ubc7 and Doa10 appear to be localized to the nuclear envelope and endoplasmic reticulum [9].

The studies of Laney and Hochstrasser [5] illustrate the importance of rapidly eliminating pre-existing regulatory components in order to effect a change of cell state. This conclusion perhaps should not be a surprise, especially as many transcription factors are intrinsically unstable [11]. More generally, post-transcriptional instability is a hallmark of critical regulatory factors, both at the mRNA and protein level [8]. This attribute surfaces time and again in biology, whether it be in developmentally regulated transcriptional programs, signal transduction or cell-cycle control. In perhaps the most dramatic and best-studied examples, the rapid destruction of cyclins and various inhibitory factors underlie each major cell-cycle transition [12].

The regulation of protein stability can also contribute to appropriate subcellular localization. For example, in another thread of the mating type tapestry, a repressor of *HO* called Ash1 is asymmetrically localized to daughter cells upon division, thereby explaining why only mothers are able to switch [1,13]. This asymmetry is achieved by transport of *ASH1* mRNA to the growing bud of the new daughter cell [14,15], coupled with inefficient translation [16] and phosphorylation-dependent proteolysis of Ash1 in newly formed mother cells ([17] and Q. Liu and M.T., unpublished data). Similar mechanisms generate axes of polarity during metazoan development. For example, in the nematode *Caenorhabditis elegans*, the PAR-1 protein localizes to the posterior cortex to help establish asymmetry of the germ plasm

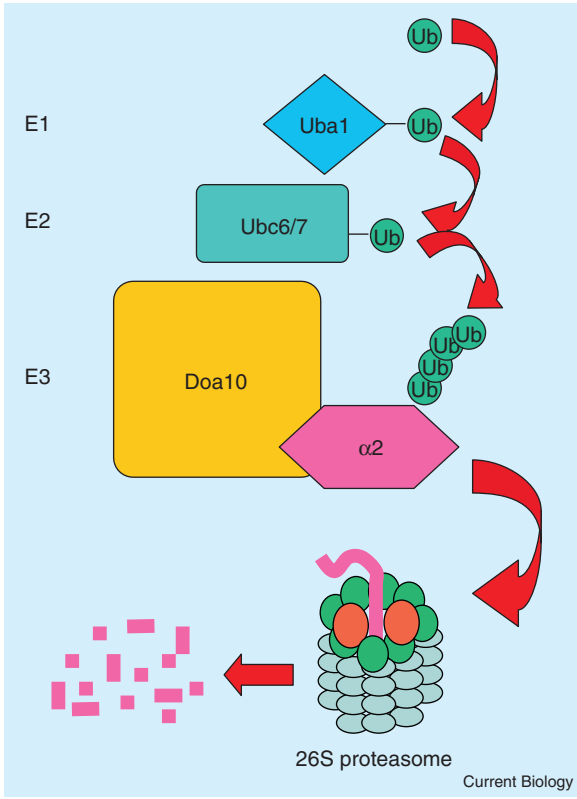

Figure 2. Ubiquitin-mediated degradation of the α2 repressor.
Ubiquitin is sequentially transferred along an E1→E2→E3 cascade and then covalently attached to a lysine residue of the α2 protein in an isopeptide linkage. Reiteration of the cycle results in substrate polyubiquitination and degradation by the 26S proteasome. An additional pathway for degradation of α2 that depends on Ubc4/5 and an unknown E3 is not shown.

in the zygote [18]. PAR-1 in turn blocks the ubiquitin-dependent degradation of PIE-1, the inhibition of which disrupts zygotic asymmetry [19]. Analogous mechanisms that dictate the instability of various master regulators will undoubtedly be at play in stem cells, which must devolve along multiple developmental lineages (for example [20]). When it comes to change in biology, the tempo seems certain to be *allegro molto*, whether it be for the violin or the orchestra.

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